THE LOCATION OF FACTORS OF HEAD REGENERATION IN THE EARTHWORM [1]

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Introduction

Common earthworms, within a certain limited extremely anterior region, can completely regenerate lost segments. At successively more posterior segments, the capacity for regeneration of characteristic head structures diminishes. Within a certain limited approximately middle region of the worm, a "tail" usually develops at a posterior cut surface, and a tail in reversed orientation (heteromorphic) may be produced at an anterior surface, but simple transection is never followed by development of a head. In the posterior region of the worm regeneration of a tail occurs at only the posterior cut surface.

These two questions arise. What determines regeneration? Is it a function of all of the organs and tissues present at the cut surface, or is some one part or group of parts responsible for the occurrence and character of regeneration? The discovery of "organizers" in embryonic development suggests the search for localized agencies determining regeneration.

A little analysis of the problem recognizes three major parts of the animal, in one or more of which the determining agencies may be seated: (1) the body wall, consisting mainly of epidermis (ectoderm) and muscle; (2) the digestive tube, including an internal epithelium and a muscle layer; (3) the central nervous organ. It seems unlikely that vascular organs or connective tissue should be directly concerned.

The central nervous structures have long been suspected of having some peculiar importance in relation to the occurrence and nature of regeneration. This has been investigated by Morgan (1902), Goldfarb (1909, 1914), Siegmund (1928), Avel (1932), Crowell (1937), and Bailey (1930, 1939). Very little has been said about the anterior body wall except that its epidermis may contribute to the regeneration of the new nervous parts. Among those who have taken a stand either for or against the epidermal origin of nervous parts are Hescheler (1898), Rand (1901), Nuzum and Rand (1924), Bailey (1930) and Schwartz

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(1932). Morgan (1902), Hunt (1919), and Nuzum and Rand (1924) have dealt with the part played by the digestive tube.

The experiments described in the present paper were designed to test body wall, digestive tube, and nerve cord as to their relation to the initiation of regeneration and the kind of structure regenerated. The method consisted in transferring (by transplantation operations) one or two of the three parts in question from the anterior head-regenerating region to the more posterior tail-regenerating region, or in the reverse direction. The general idea was to make all possible combinations of body wall, gut, and nerve cord of head-forming and tail-forming regions of the worm.

Materials and Methods

The common earthworm, *Allolobophora caliginosa,* was used in all experiments. Among those used were a few (perhaps six) more highly pigmented than the others. Except for the pigment, however, no difference could be detected.

Previous to operation, the worm was anesthetized by placing in 0.2 per cent chloretone for about ten minutes. It was then laid on a glass plate covered by a wet paper towel. A simple tripod lens, or in difficult cases a binocular dissecting microscope, was used. Ordinary small scissors and forceps, sometimes jeweler's forceps, were adequate. Especially useful were sharpened needles inserted into large match sticks.

After the transplantation, stitches were taken with the use of a small needle (No. 12), and one strand of a fine three-strand silk thread. The worm was then placed in a glass tumbler between layers of wet paper toweling. The tumblers, each covered by a square of glass, were kept in a cold room at 3° C. for three or four days and then moved to a water-table where the temperature was about 7° C. This temperature was maintained for nearly three weeks. Following this, room temperature was considered most favorable for regeneration. At the desired stages, the worms were fixed in Bouin's picro-formol solution, serially cut in sagittal plane at 8 μ, and stained in Ehrlich's hematoxylin and eosin.

Experimental Work

Following is an outline of the several types of experiments employed:

Series A. Digestive Tube.

Type 1. Gut and nerve cord of a region behind clitellum removed and pharynx implanted.

Type 2. Gut and nerve cord of a region behind clitellum replaced respectively by pharynx and anterior nerve cord.

Type 3. Gut of a region behind clitellum replaced by pharynx; posterior nerve cord left in position.

Series B. Nerve Cord.

Type 1. Nerve cord of a region behind clitellum replaced by nerve cord from head-forming region.

Type 2. Nerve cord in head-forming region replaced by nerve cord from tail-forming region.

Series C. Body Wall.

Type 1. Segments from head-forming region, including gut but lacking the central nervous organs, sewed in normal antero-posterior orientation to anterior surface of a post-clitellar segment; protruding nerve cord of host component pulled into graft.

Type 2. Anterior body-wall rings from head-forming region slipped over intestine back of clitellum ("sleeve operation"):

(*a*) no central nervous organs present within graft;

(*b*) anterior nerve cord present within graft;

(*c*) posterior cord drawn forward into graft;

1. "sleeve" placed in normal orientation,
2. "sleeve" placed in reversed antero-posterior orientation.

The experiments in which rings of post-clitellar body wall were grafted over the pharynx are not included in the above outline. The operations were unsuccessful, due in large part to the stretching undergone by the posterior body-wall rings as they were pulled over the bulky pharynx. The single animal which survived the above operation gave no evidence of the initiation of either head or tail regeneration. A dozen other cases in which the pharynx was replaced by intestine from a post-clitellar region are omitted from the outline because none survived.

The post-clitellar region to which pharynx, anterior nerve cord, or anterior body wall was transferred lies at various levels between the fifth and twentieth segments behind the clitellum. From this same region parts were transferred to the anterior head-forming region of the worm. The work of Crowell (1937) showed that this post-clitellar region is capable of giving rise to a normal tail in a posterior direction, and a heteromorphic tail in an anterior direction. A series of controls set up by simple transection five, ten, and fifteen segments behind the clitellum verified Crowell's findings. This region was selected, therefore, because in normal regeneration it never produces anything except a tail.

The text figures illustrate not only the several types of experiments employed, but also particular cases which are to be considered as representative of the types. The plate figures are from photographs of sections, and should be compared with the diagrams of the results, shown in the text figures. In the case histories such expressions as "segments 4–11" are always to be understood as including the two segments whose numbers are given.

The text figures are diagrams of median longitudinal sections of the earthworm. The arrows indicate the source and new site of transferred parts and also the discarding of parts. The body wall is stippled; the nervous organs are in solid black. In the diagram representing the result of an experiment the wall of the digestive tube is cross-hatched. Omission of segments is designated by the irregular breaks in the diagrams.

Abbreviations:

A. N.c. ..... anterior nerve cord
C. ..... clitellum
I. N.c. ..... implanted nerve cord
Int. ..... intestine
P.-c. ..... post-clitellar
Ph. ..... pharynx
P. N.c. ..... posterior nerve cord
R. N.c. ..... regenerated nerve cord

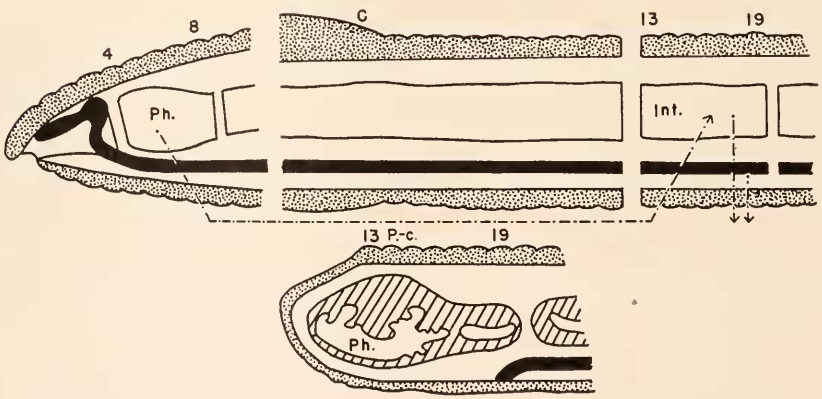

FIG. 1. The pharynx and oesophagus of segments 4–8 replace the intestine and nerve cord of post-clitellar segments 13–19. Result, shown below, is negative.

Series A. Digestive Tube

Type 1. Gut and nerve cord of a region behind clitellum removed and pharynx implanted.

Case 77 (*Figs. 1 and 12*).—The intestine and nerve cord of post-clitellar segments 13–19 were removed. To this position were transferred, in normal orientation, the pharynx and oesophagus of segments

4–8. The injured head region was then removed. After healing of the posterior incision through which the implant had been inserted, transection was made between the twelfth and thirteenth post-clitellar segments. The worm was fixed at the end of three and one-half months.

Although well oriented with reference to adjacent parts, the pharynx does not open to the outside (Figs. 1 and 12). The nerve cord, turned toward the ventral body wall, reveals little or no growth activity at its anterior end.

In most of the other cases of this type the transection following the healing of the longitudinal incision was made in such a way as to remove the anterior third of the pharynx. It was felt that establishing a freshly cut surface of the pharynx as well as of the body wall would be more conducive to regeneration. In all cases, however, the wound-surface healed over smoothly and no regeneration occurred.

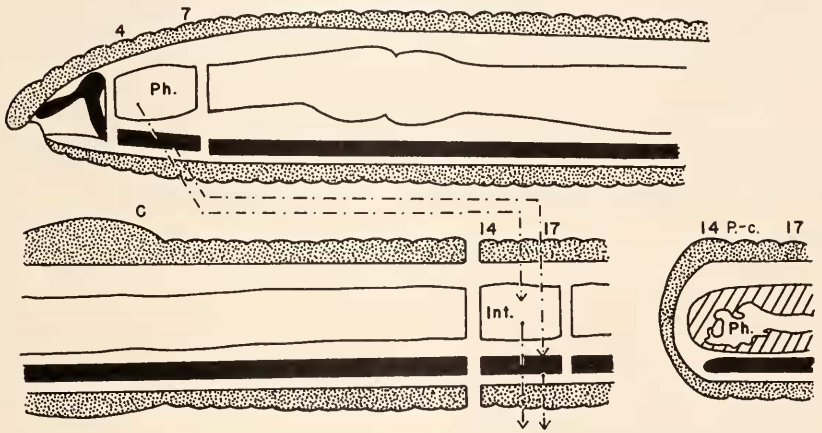

Fig. 2. The pharynx, oesophagus, and nerve cord of segments 4–7 replace the intestine and nerve cord of post-clitellar segments 14–17. Result, negative.

Type 2. Gut and nerve cord of a region behind clitellum replaced by pharynx and anterior nerve cord.

Case 11 (Figs. 2 and 22).—The intestine and nerve cord of post-clitellar segments 14–17 were removed. In their place were implanted, in normal orientation, the pharynx and a portion of the oesophagus together with the nerve cord from segments 4–7. After healing, transection was made at such a level as to remove a small part of the implanted pharynx. The remainder protruded to some extent. At the end of a month, the worm was fixed.

Except for a little proliferation at the forward end of the implanted cord, the result is negative (Figs. 2 and 22). Although communication between the oesophagus and the intestine was established, no anterior perforation to the outside occurred; the cut surface healed over smoothly.

An exceptional case (No. 84, Figs. 20 and 21) of this same type requires special attention. The implant, consisting of pharynx, oesophagus and nerve cord from segments 4–8, replaced the intestine and nerve cord of post-clitellar segments 13–23. After healing, transection was made immediately anterior to the implant. At the end of two and one-

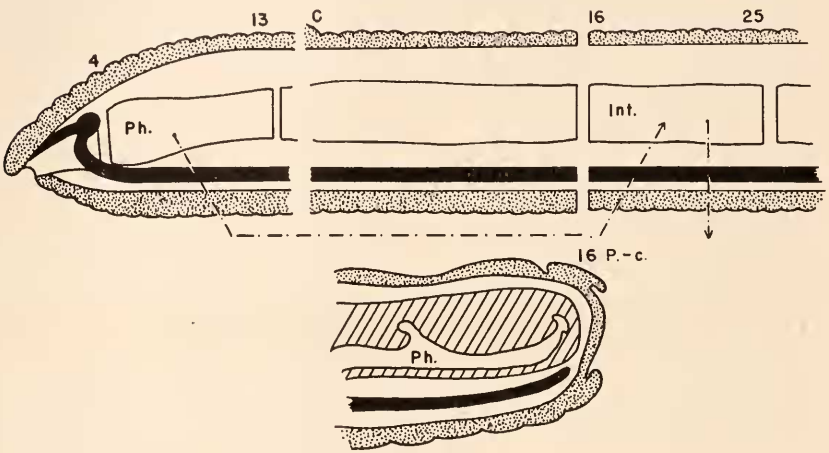

Fig. 3. With posterior nerve cord left in position, the intestine of post-clitellar segments 16–25 is replaced by pharynx and oesophagus of segments 4–13. Result, negative. The antero-posterior orientation of the lower part of the diagram is reversed to correspond to that of Fig. 16.

half months the worm was fixed. The sections (Figs. 20 and 21) show a well-formed brain, and commissures extending down to the nerve cord. Communication between the oesophagus and the intestine was established, but no anterior opening from the pharynx to the outside. The outstanding feature of the case is the close relationship between the nervous tissue and the pharynx epithelium (Fig. 21).

Type 3. Gut of a region behind clitellum replaced by pharynx; posterior nerve cord left in position.

Case 68 (Figs. 3 and 16).—The pharynx and oesophagus from segments 4–13 replaced, in normal orientation, the intestine of post-clitellar segments 16–25. The posterior nerve cord was left in position.

After healing of the longitudinal incision, transection was made between the fifteenth and sixteenth post-clitellar segments. Two and one-half months later the worm was fixed.

The sections reveal little cellular activity other than wound closure and healing. Certain sections show some proliferation of cells from the anterior end of the nerve cord, but give little evidence that a brain might have regenerated.

Series B. Nerve Cord

Type 1. Nerve cord of a region behind clitellum replaced by nerve cord from head-forming region.

Case 60 (Figs. 4 and 23).—The nerve cord of post-clitellar segments 10–17 was replaced by the anterior nerve cord from segments 4–11.

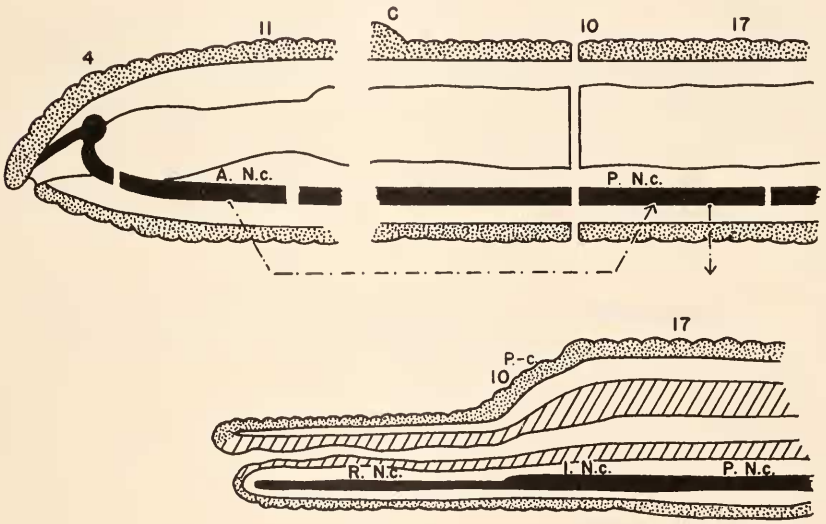

Fig. 4. Nerve cord of post-clitellar segments 10–17 is replaced by anterior nerve cord of segments 4–11. Result, heteromorphic tail. Implanted anterior cord has made connection with posterior cord.

The implant was made in normal orientation. After the healing of the posterior incision, simple transection was made just anterior to it, between post-clitellar segments 9 and 10. Examination at the end of two and one-half months reveals the presence of a heteromorphic

tail of about twenty-five segments. New nervous material extends to the tip of the regenerate. Because of the bending of the worm, no one section shows both the anus and portions of the new and the implanted nerve cord.

Type 2. Nerve cord in head-forming region replaced by nerve cord from tail-forming region.

Case 61 (*Figs. 5 and 13*).—A median longitudinal incision was made on the ventral surface of the thirteenth and fourteenth anterior segments. This permitted severing the cord between these two segments. Anterior to the fifth anterior segment the cord was grasped

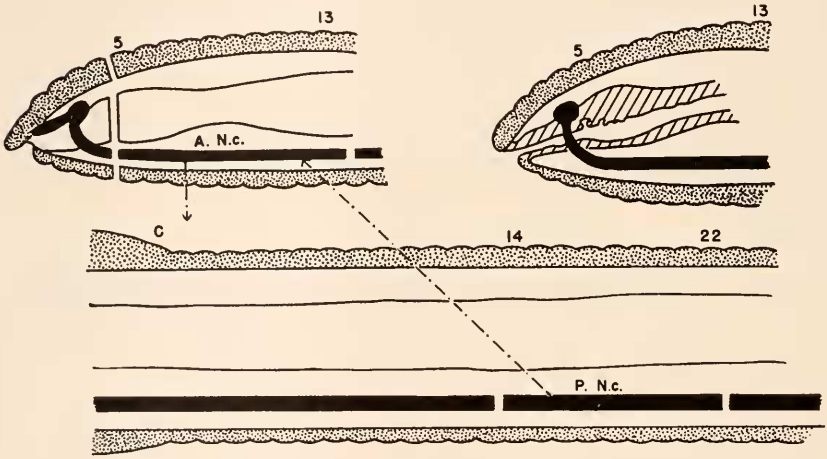

Fig. 5. Anterior nerve cord of segments 5–13 is replaced by nerve cord of post-clitellar segments 14–22. The first four segments are discarded. A new brain, commissures, three new segments, and a prostomium are regenerated.

firmly and pulled from segments 5–13. The nerve cord from post-clitellar segments 14–22 was pulled, in normal orientation, into these anterior segments by the use of silk thread and a long needle. The first four head segments, including the nervous organs, were discarded. At the end of two and one-half months the worm was fixed.

A new head with brain, commissures, and oral opening is evident. Three segments and a prostomium are new. The brain lies in the third new segment.

In Case 103, the removal of the anterior nerve cord from segments

6–12 was following by the implantation, *in reversed orientation,* of the nerve cord from post-clitellar segments 13–27. In two and one-half months a brain, lacking commissures, formed independently of the implanted cord. An anterior perforation of the gut to the outside did not occur.

Series C. Body Wall

Type 1. Segments from head-forming region, including gut but lacking the central nervous organs, sewed in normal antero-posterior orientation to anterior surface of a post-clitellar segment; protruding nerve cord of host component pulled into graft.

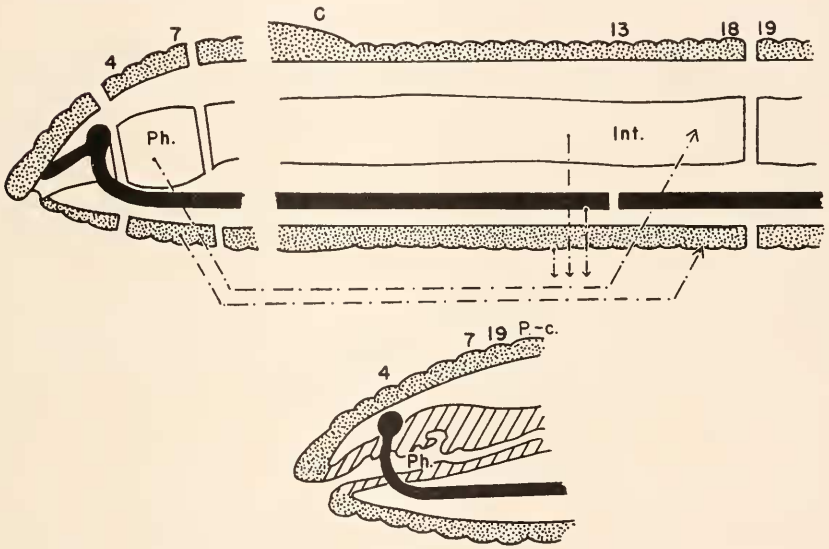

FIG. 6. Anterior segments 4–7, lacking the nervous parts, are grafted, in normal orientation, to anterior face of nineteenth segment behind the clitellum. The posterior nerve cord is drawn into the graft. Complete head regeneration results.

Case 47 (Figs. 6 and 18).—The anterior segments 4–7, including the digestive tube, but lacking the nerve cord, were sewed in normal orientation to the anterior face of the nineteenth post-clitellar segment. The nerve cord of post-clitellar segments 13–18 was pulled into the graft in the position occupied formerly by the anterior nerve cord. Two and one-half months later the worm was fixed.

Investigation reveals a regenerate possessing two segments, a prostomium, an oral aperture, a brain located in the third segment (first

of the graft or fourth of the original worm), and commissures extending down to the nerve cord. The pharynx communicates with the intestine by means of a short portion of oesophagus.

In this case occurs the same combination of parts as in Series B, Type 2. In both, the posterior nerve cord participates in head regeneration.

Type 2. Anterior body-wall rings from head-forming region slipped over intestine back of clitellum ("sleeve operation");

(*a*) No central nervous organs present within transplant ("sleeve").

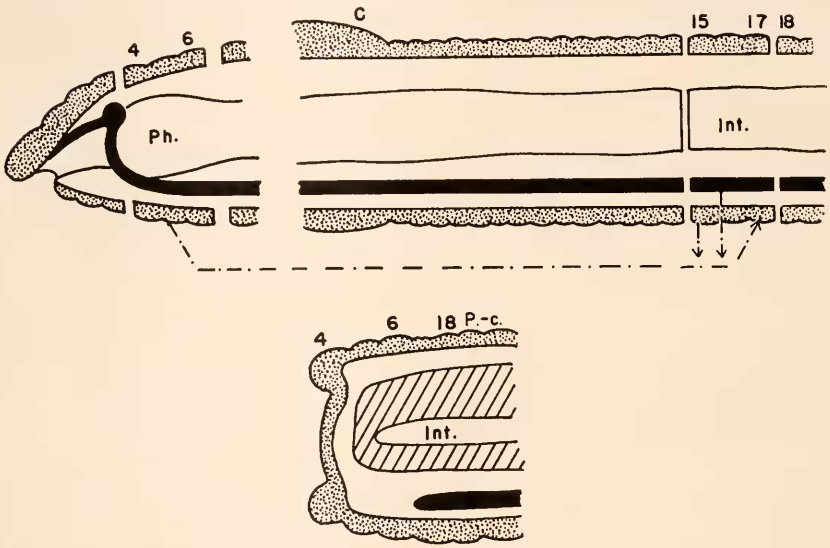

Fig. 7. A sleeve of anterior body-wall segments 4–6 is grafted over intestine of post-clitellar segments 15–17. The operated region contains no central nervous parts. Result negative.

Case 37 (*Figs. 7 and 15*).—A sleeve consisting of anterior body-wall segments 4–6 was slipped over the intestine of post-clitellar segments 15–17. All central nervous parts in the region of operation were discarded. The sleeve was normally oriented and sewed to the anterior face of the eighteenth post-clitellar segment. At the end of two months the worm was fixed. The sections show no activity except wound closure and healing.

In Case 17 the resulting conditions are somewhat different. The operation was the same as that above (Case 37) except that the sleeve was slipped over the intestine of post-clitellar segments 12–14 instead of 15–17. The length of time between operation and fixation was the same in both. In Case 17, however, a brain formed without any relation whatever to the ventral nerve cord. No commissures were evident; there is no anterior opening of the gut to the outside.

Cases 78 and 79 differ from both of the preceding in that the posterior nerve cord grew forward almost to the end of the sleeve.

Except these last three cases, all of this type agree in result with that of Case 37.

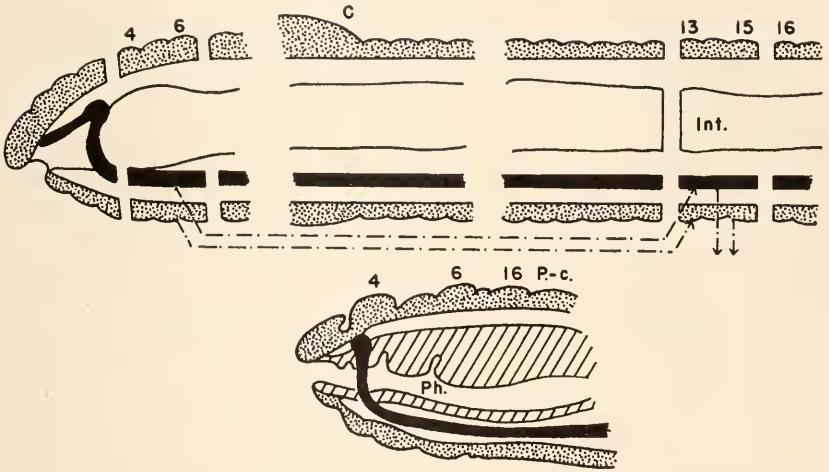

FIG. 8. A sleeve of anterior body-wall segments 4–6, including nerve cord, is grafted over intestine of post-clitellar segments 13–15. Head regeneration results.

(*b*) Anterior nerve cord present within graft.

Case 65 (*Figs. 8 and 14*).—After discarding the body wall and nerve cord of post-clitellar segments 13–15, a sleeve of anterior body-wall segments 4–6, together with their nerve cord, was slipped over the protruding intestine and attached (by stitches) in normal orientation to the anterior face of the sixteenth post-clitellar segment. At the end of two and one-half months the worm was fixed. Sections (Fig. 14) show a well-formed brain, commissures, typical pharynx epithelium, and an anterior opening of the gut to the outside.

Case 42 (Fig. 11) shows a regenerating brain and commissures, and an imperforate stomodeum. Figure 10, from another section of the same worm, shows the close relationship between the newly-formed brain and the epidermis.

(*c*) Posterior cord drawn forward into graft.

1. "Sleeve" placed in normal orientation.

Case 27 (*Figs. 9 and 19*).—A sleeve of anterior body-wall segments 4–6 was slipped, in normal orientation, over the intestine and nerve cord of post-clitellar segments 10–12. At the end of two months the

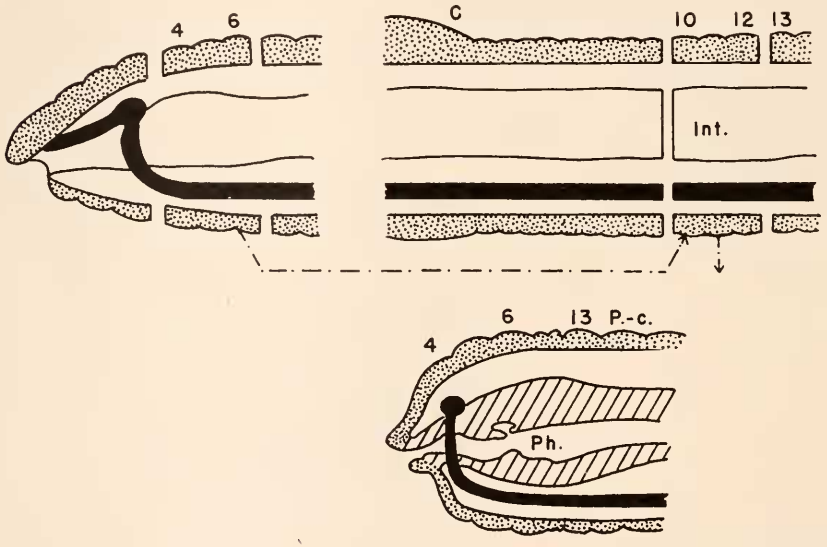

FIG. 9. A sleeve of anterior body-wall segments 4–6 is grafted over intestine and nerve cord of post-clitellar segments 10–12. Result, head regeneration.

worm was fixed. Sections show a new head with brain, commissures, a small amount of differentiated pharynx epithelium, and an oral opening.

With the exception of the oral opening, Case 32 possesses all the regenerated head parts noted in Case 27. The question of the origin of the pharynx epithelium will be discussed in another section of the paper.

Case 74 is another which clearly demonstrates the ability of the posterior nerve cord and intestine to participate in head formation when combined with anterior body wall.

The results of the three cases cited above, which agree essentially with those of seven additional cases, appear in Table I.

Three variations in the operative procedure, not included in Table I, showed different results. In Cases 75 and 83 the sleeve was oriented so that its dorsal side coincided with the ventral side of the host. As a

TABLE I

Explanation of Symbols

	Anterior	Posterior
Body Wall	W	w
Gut	G^p (pharynx)	g^i (intestine)
Nerve Cord	N	n

	Combination	Number of Cases	Result
Series A			
Type 1	wG^p	13	No regeneration
Type 2	wG^pN	8	No regeneration
		1	Head
Type 3	wG^pn	5	No regeneration
Series B			
Type 1	wg^iN	7	No regeneration
		1	Heteromorphic tail
Type 2	WG^pn	6	Head
Series C			
Type 1	WG^pn	5	Head
Type 2			
(a)	Wg^i	3	No regeneration
		1	Brain only
		2	Proliferation at end of nerve cord
(b)	Wg^iN	3	Head
(c)			
(1)	Wg^in normal orientation	10	Head
(2)	Wg^in W reversed antero-posteriorly	2	Results negative. Figure 17 shows slight regeneration.

result, the posterior nerve cord, pulled into the dorsal side of the sleeve at the time of operation, became attached dorsally by strands of connective tissue. No regeneration occurred.

In Case 44 the sleeve was oriented so that its dorsal side was lateral with reference to the dorsal side of the host component. At the end of a month and a half the posterior nerve cord had grown laterally to meet or give rise to new nerve material lying in the lateral plane (dorsal portion of sleeve).

2. "Sleeve" placed in reversed antero-posterior orientation.

Case 72 (*Fig. 17*).—A sleeve of anterior body-wall segments 4–6 was slipped over the intestine and nerve cord of post-clitellar segments 25–27. In contrast to previous cases, however, the sleeve was reversed so that segment 4 of the sleeve was sewed to the anterior face of post-clitellar segment 28. This left segment 6 of the graft in the anterior position. Normal dorso-ventral orientation of the graft was preserved. At the end of two and one-half months the worm was fixed.

Figure 17 shows a very slender anterior regenerate in the form of a finger-like projection. The nerve cord has grown forward and enters the base of the projection.

The results in this and in the one other case are the same; the activity of the nerve cord is confined entirely to the ventral side.

Out of more than four hundred worms used in the various experiments, only a small proportion survived. The significant results are summarized in Table I.

Discussion

The Rôle of the Digestive Tube in Regeneration

No experiment was performed with the express purpose of finding out whether or not regeneration would occur in the absence of the digestive tube at the wound region. The work of Morgan (1902) and Hunt (1919) shows that a head may begin to regenerate before the digestive tube, removed from the region just behind the plane of transection of the body, has grown forward to the wound region. In all the experiments performed in the present study some part of the digestive tube was present at the wound surface.

The pharynx alone, or in conjunction with the posterior nerve cord, failed to evoke head regeneration when implanted back of the clitellum. In like manner, the cases involving the combination of pharynx and anterior nerve cord in the posterior position are negative with the exception of Case 84. In this case, as already stated, there is a close relationship between the new nervous tissue and the pharynx epithelium (Figs. 20, 21). The characteristic columnar condition of the epithelium in this region has been lost. The layer, lacking a basement membrane, is made up of flattened cells with large, spherical nuclei. These epithelial cells merge uninterruptedly into the nerve cells so that there is no sharp demarcation between the epithelial layer and the brain anlage. Mitoses are abundant. There is very little evidence of proliferation

from the old nerve cord. The relatively great distance of the epidermis from the brain (Fig. 20), and the absence of mitoses in the epidermis preclude any idea of contribution to brain formation from this source. The similarity of the pharynx epithelial and the prospective brain cells, their close proximity, and the abundance of mitotic figures indicate that, in this case at least, the pharynx epithelium has contributed to brain formation.

The apparent manner of origin of the brain in Case 84 is very similar to that described by Nuzum and Rand (1924). As early as a week following removal of the brain, without removal of any other part, they found groups of deeply-staining cells lying just above and in close relation to the pharynx epithelium. According to them, "These cells, containing large nuclei with prominent nucleoli, are similar to the cells of both pharyngeal and epidermal epithelia. Their position, corresponding precisely to that occupied by the obvious brain fundament of later stages, as well as their character, marks them as the early brain fundament" (p. 218). Siegmund (1928) suggested that the pharynx may be concerned in the formation of a brain which arises independently of the nerve cord.

It is possible, then, that the pharynx, ectodermal in ontogeny, may have some head-determining capacity. Results obtained in Series C, Type 2, show that the intestine can participate in head regeneration (Figs. 11, 14, 19), and that the epithelium of the intestine can dedifferentiate and redifferentiate into pharynx epithelium. Case 32 gives evidence that the new pharynx epithelium is derived from the intestine since there is no oral perforation and hence no possibility of contribution to pharynx formation by stomodeal epithelium. The endodermal origin of pharynx epithelium in regeneration is described by Hescheler (1898, p. 581): "Das regenerierte Pharynxepithel geht aus den Zellen des alten Darmes hervor." Apparently, then, the pharynx is not essential to head regeneration even though it may, under some circumstances, be capable of giving rise to a brain.

The Rôle of the Nerve Cord in Regeneration

Morgan (1902, p. 571) maintained that the "cut-end of the nerve-cord is necessary at a cut-surface in order that a new head may develop." Bailey (1930) observed that regeneration does not occur when the nerve cord is looped back so that its cut end is remote from the wound. Results obtained by Goldfarb (1914), Siegmund (1928), Avel (1932), Crowell (1937) and Bailey (1939) show that the presence of the nerve at the wound surface is not essential to head regeneration; a brain may form

independently of primary connection with the nerve cord. In the present work two instances of this sort, described above, were obtained. In Case 17, the sleeve of anterior body wall, grafted over the intestine back of the clitellum, contained neither anterior nor posterior nerve cord, yet a new brain formed in the anterior segment of the sleeve. Its close relation to the epidermis and the similarity between the cells of the brain anlage and the flattened epidermal cells give evidence that this brain was derived from the epidermis of the transplant.

Case 103 consisted of the transplantation of posterior nerve cord, in reversed orientation, to the head-forming region. As in Case 17 above, a brain formed showing close relationship to the epidermis but having no cellular connection with the implanted cord.

In all such cases where a brain develops independently of cellular connection with the nerve cord, there is the possibility that the cord exerts an influence through some neurohumor-like substance not observable in histological studies.

Especially significant are the sleeve cases and the cord transplantation experiments which demonstrate that the posterior nerve cord, usually associated only with regeneration of normal or of heteromorphic tails, can participate in head regeneration, and that the anterior nerve cord from the head-forming region can participate in the formation of a heteromorphic tail. Nervous structures of new heads in whose regeneration either anterior or posterior cord has participated show evidence of cellular proliferation in the old nerve cord. It is evident, however, that the nerve cord does not determine the form of the regenerate, and so can not be of the nature of an organizer.

The importance of the part played by the brain cannot be denied. The establishment of a brain anlage is always the first event in head regeneration. If, however, the nerve cord is concerned in this establishment, the posterior nerve cord is just as effective as the anterior nerve cord. We must look elsewhere for a specific *head* determiner. As suggested by Crowell (1937), it is not improbable that the new brain, however determined itself, may act as organizer during the further course of head regeneration.

The Rôle of the Anterior Body Wall in Regeneration

According to Hescheler (1898, p. 580) the epidermis is the chief source of cells giving rise to regenerated nervous material. He reports: "Während der ganzen Dauer der Bildung des neuen Centralnervensystems war keine grössere zusammenhängende Abspaltung von Epider-

miszellen, sondern nur Zuwanderung einzelner Zellelemente zu beobachten." While Rand (1901) agrees with Hescheler that the epidermis is an important source of nervous material, Bailey (1930) observes no cells coming from the epidermis, and Schwartz (1932) claims that the connective tissue is the source of new cells in the cerebral ganglia. The observations made in the present study agree with those of Hescheler and Rand.

The rôle of the body wall is clearly seen in those cases where a sleeve of anterior body wall is grafted into the post-clitellar region in normal orientation with relation to the host component. A combination of such a sleeve, either anterior or posterior nerve cord, and intestine always evokes head regeneration. Attention has been called to Figure 17 (Case 72) which shows the negative results obtained when a sleeve of anterior body wall is grafted in reversed polarity over intestine and posterior nerve cord. This case, as well as the one other, involves the same combination of parts which, when all are in normal orientation, always produces a head.

Most of the normally oriented cases studied in this work represent such an advanced condition of regeneration that it is difficult to ascertain by what method the sleeve determines the form of the regenerate. Two cases, representing early stages in head regeneration, will be considered in more detail.

Case 17 was discussed briefly in connection with the rôle played by the nerve cord. The sleeve of anterior body-wall segments, enclosing the intestine back of the clitellum, contained neither anterior nor posterior nerve cord. The anterior end of the sleeve, at first open, closed in healing. The anterior cut end of the intestine likewise closed. Between the healed and closed anterior ends of body wall and intestine lies a cicatricial mass in which is embedded a brain anlage lying close to the epidermis. In the vicinity of the brain the epidermal layer, made up of flattened cells, is thinner than elsewhere. It is concave toward the brain, suggesting the beginning of a stomodeal invagination. The cells of the brain anlage, epidermis, and thin intervening mass are similar, all being of the epithelial type with large spherical nuclei and prominent nucleoli.

Case 42 (Figs. 10 and 11), in which an anterior body-wall sleeve and anterior nerve cord were grafted over the intestine, shows a developing brain, commissures, and an imperforate stomodeum. The relation between the brain and an inpocketing of the epidermis is seen in Fig. 10. Although ordinarily made up of columnar cells, the epidermal layer in this region consists of flattened cells and, furthermore, lacks the usual

basement membrane. The cells appear to be undergoing a process of redifferentiation, a process marked by active mitosis and a distinct migration of the newly-formed cells to constitute the regenerating brain. This migration gives, in places, the effect of "streaming" of cell masses. The evidence afforded by this and the preceding case makes it clear that the epidermis of the anterior body wall initiates head regeneration by contributing cells which go to make up the brain. The fact that head regeneration has never been observed to take place in the absence of a brain anlage indicates that the brain, once established, evokes further and complete development of the head.

The question may now be asked, in what region of the anterior body wall is located the seat of brain determination, the dorsal portion, the ventral, or the lateral portion? With the exception of number 84, in which the brain anlage bears a close relation to the pharynx epithelium, all cases in which brain formation has been described indicate that the dorsal portion of the body-wall sleeve is the site of determination. In cases 75 and 83 the sleeve, ordinarily dorso-ventrally oriented with reference to the host, was oriented so that its dorsal side coincided with the ventral side of the host. This resulted in the apposition of the end of the posterior nerve cord and ventral side of the intestine against the dorsal side of the sleeve. Although giving no evidence in favor of the dorsal side as the region of determination, the fact that no regeneration occurred suggests that the ventral side of the sleeve, placed in the dorsal position, plays no rôle in the initiation of brain formation.

In Case 44 the sleeve was oriented so that its dorsal side was lateral with reference to the dorso-ventral axis of the host. Sections reveal that the posterior nerve cord has grown forward and laterally from its ventral course to meet or give rise to new nerve material lying in the lateral plane (dorsal part of sleeve). If the new material comes partly or even entirely from the old cord, then apparently the direction of its regeneration has been determined by the misplaced dorsal side of the graft. If the new material has its origin in the dorsal region of the graft, it and the old cord have advanced toward each other and united. According to Joest (1897), if an earthworm be transected and the two parts grafted together so that they are not properly oriented, the ends of the nerve cord, if not too far apart, will grow toward each other and re-establish connection.

In conclusion, it may be said that, in head regeneration, the brain, once established by the epidermis of the dorsal body wall, takes over the direction of further head development including the establishment of commissures and their connection with the nerve cord. We may thus regard the dorsal epidermis of the anterior body wall as the primary head determiner, and ascribe to the brain the rôle of secondary organizer.

Summary

1. The presence of pharynx at a wound region is not essential to head regeneration even though it may, under some circumstances, be the source of nerve material (brain).

2. While obviously not a head determiner, the intestine can participate in head regeneration apparently as readily as the pharynx can. Intestinal epithelium can redifferentiate into characteristic pharynx epithelium.

3. The presence of nerve cord at a cut surface is not an essential factor in head regeneration since a brain may develop independently of any cellular connection with the cord. There may, however, be some hormonal relation between the nerve cord and regeneration.

4. The nerve cord does not determine the type of the regenerate for:

(*a*) The anterior (cephalic) nerve cord can participate in heteromorphic tail formation as well as in head formation.

(*b*) The post-clitellar cord (within the first twenty-five segments behind the clitellum) can participate in head formation as well as in normal and heteromorphic tail formation.

5. The "sleeve" operation, consisting in grafting over the intestine, in normal orientation, segments of anterior body wall containing either no central nervous parts, or anterior or posterior nerve cord, was invariably followed by head regeneration except in most of those cases in which no nerve cord was initially present. The "sleeve" cases afford strong evidence that the epidermis of the anterior body wall, being the source of the brain anlage, contains the primary determiner of head regeneration, and that the seat of this determination is probably located in the dorsal part of the epidermis.

Acknowledgment

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PLATE I

Explanation of Figures

Abbreviations used in plate figures:

Br. ..... brain
B.v. ..... blood vessel
I. N.c. ..... implanted anterior nerve cord
Int. ..... intestine
N.c. ..... nerve cord
Ph. ..... pharynx
R. N.c. ..... regenerated nerve cord

10. Case 42. High-power view of brain a few sections from that shown in Fig. 11 of the same case. Note proximity of brain to inpocketing epidermis. × 290.

11. Case 42. Regenerating head resulting from combination of a sleeve of anterior body-wall segments (4–6), anterior nerve cord, and intestine. Note imperforate stomodeum. × 29.

12. Case 77. Pharynx implanted thirteen segments behind the clitellum. No nerve cord within operated region. No regeneration. × 34.

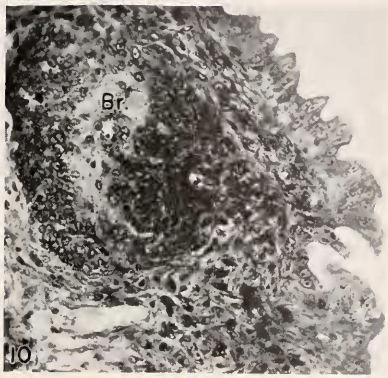
13. Case 61. Head regeneration occurred when posterior nerve cord replaced anterior cord. × 35.

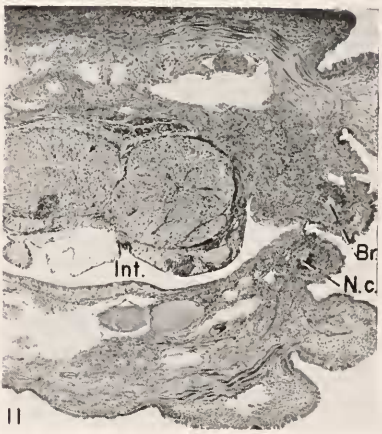
14. Case 65. Regeneration of a head following graft of sleeve of anterior body-wall segments (4–6) and anterior nerve cord over intestine. Other sections show stomodeal perforation complete. ×31.

15. Case 37. Sleeve of anterior body-wall segments (4–6) grafted over intestine. No nervous parts included. Regeneration did not occur. × 24.

16. Case 68. Pharynx implanted back of clitellum. Posterior nerve cord left in position. No head regeneration. × 36.

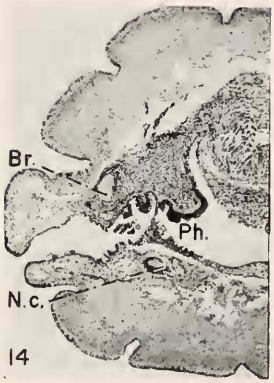
17. Case 72. Sleeve of anterior body-wall segments (4–6) grafted in reversed antero-posterior orientation over intestine and nerve cord back of clitellum. No head regeneration. × 33.


Br.
10


Int.
Br.
N.c.
11


Ph.
12


Br.
Ph.
13
N.c.


Br.
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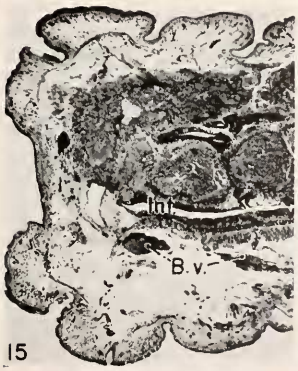
14


Int.
B.v.
15


Ph.
N.c.
16

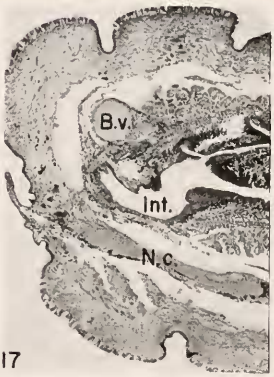

B.v.
Int.
N.c.
17

PLATE I

PLATE II

Explanation of Figures

18. Case 47. Regeneration of head following graft of anterior segments (4–7) to posterior portion of worm. Posterior nerve cord drawn into graft, replacing anterior cord. × 33.

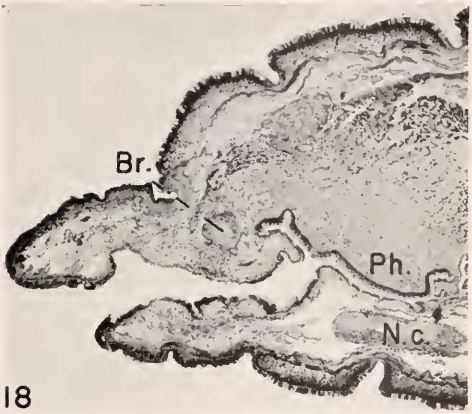
19. Case 27. Head regeneration following graft of sleeve of anterior body-wall segments (4–6) over intestine and nerve cord back of clitellum. × 45.

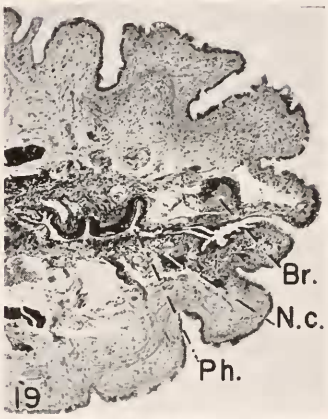
20. Case 84. Regeneration of a brain following implantation of pharynx and anterior nerve cord behind the clitellum. × 38.

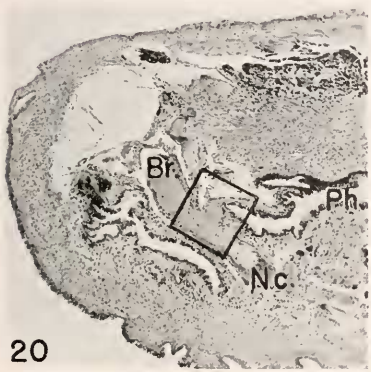
21. Case 84. Enlargement of that portion of Fig. 20 inclosed within square. Note flattening of cells of pharynx epithelium and the resemblance of its cells to the nerve cells. × 304.

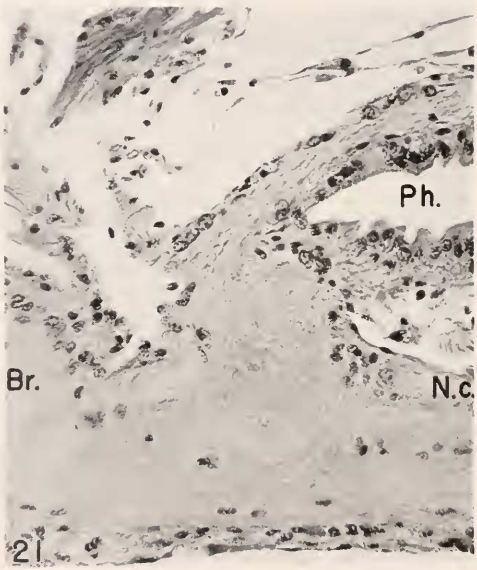
22. Case 11. Pharynx and anterior nerve cord are implanted back of clitellum. Some forward growth of nerve cord but no brain anlage. × 42.

23. Case 60. A heteromorphic tail formed when anterior nerve cord replaced posterior cord back of clitellum. × 33.









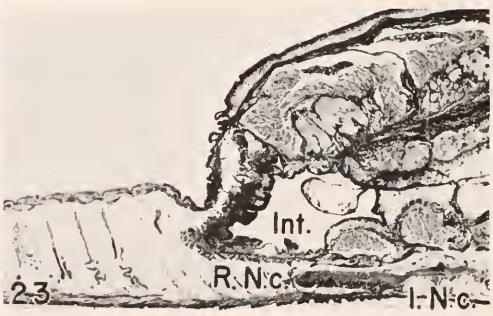


PLATE II